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Hypoglycemic effects of the wood of *Taxus yunnanensis* on streptozotocin-induced diabetic rats and its active components

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Abstract

Hypoglycemic effects of the H_2O and MeOH extracts of the wood of *Taxus yunnanensis* were examined in streptozotocin (STZ)-induced diabetic rats. The H_2O extract significantly lowered the fasting blood glucose level by 33.7% at a 100 mg/kg dose on intraperitoneal administration. From the active H_2O extract of the wood, three lignans, i.e., isotaxiresinol (**1**), secoisolariciresinol (**2**) and taxiresinol (**3**), were isolated as major components. These lignans were further tested for their hypoglycemic effects on the same experimental model. At a dose of 100 mg/kg (i.p.), isotaxiresinol (**1**) reduced the fasting blood glucose level of diabetic rats by 34.5%, while secoisolariciresinol (**2**) and taxiresinol (**3**) reduced by 33.4% and 20.9%, respectively. The blood glucose lowering effects of **1** and **2** were stronger than the mixture of tolbutamide (200 mg/kg) and buformin (1 mg/kg) used as a positive control, which lowered fasting blood glucose level by 24.0%.

Keywords: *Taxus yunnanensis*; Hypoglycemic effect; Lignan; Isotaxiresinol; Secoisolariciresinol; Taxiresinol

Introduction

Diabetes mellitus is a metabolic disorder affecting the metabolism of carbohydrate, fat and protein. It comes as a leading cause for human death and has been reported to afflict an estimated 6% of the adult population of western society and its worldwide frequency is expected to continue to grow by 6% per annum, potentially reaching 200–300 million cases in 2010 (Kahn and Shechter, 1990; Moller, 2001). Several approaches were made presently to reduce the hyperglycemia such as treatment by sulfonylureas, which stimulates pancreatic islet cells to secrete insulin; metaformin, which acts to reduce hepatic glucose production; α-glucosidase inhibitors, which interfere with glucose adsorption and insulin itself, which suppresses glucose production and augments glucose utilization (Moller, 2001). All these therapies have limited efficacy and various side effects, and thus searching for new classes of compounds is essential to overcome these problems. Several medicinal plants have been reported to be used in traditional medicine for the treatment of diabetic patients in different ethnic societies of Asia, Africa and the South America. Even in developed countries of Europe, North America and Japan, several plant products are used for the treatment

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of diabetes in the name of herbal drugs. *Taxus yunnanensis* Cheng and L. K. Fu (Taxaceae), an evergreen tree commonly known as Hongdoushan, is one of such plants and widely distributed in Yunnan Province of China (Delectis Florae Republicae Popularis Sinicae Agendae Academiae Sinicae, 1978). The wood of *T. yunnanensis* has been used in traditional Chinese medicine by the people of several ethnic societies in Yunnan Province for treatment of kidney problem and diabetes mellitus (Chiang Su New Medical College, 1977), while the bark and leaves are rich in taxane-type diterpenes including paclitaxel (Taxol®), a promising anticancer agent used clinically for the treatment of various cancer (Baloglu and Kingston, 1999). Because of its uses in traditional medicine as an antidiabetic agent, we examined the hypoglycemic activity of the wood of *T. yunnanensis* in streptozotocin (STZ)-induced diabetic rats.

Materials and methods

Chemicals and instruments

STZ and tolbutamide were purchased from Sigma Chemicals (St. Louis, USA). Glucose detecting kit (Glucose CII Test Wako) and acacia gum were from Wako Pure Chemicals Industry (Osaka, Japan). Buformin was from Japan Galen (Saitama, Japan). UV measurements were done on a Shimadzu UV-160A UV–Visible spectrophotometer. HPLC was performed in Shimadzu LC-6AD using discovery C18 column (4.6 mm i.d. × 25 cm, Supelco, USA). The mobile phase was H_2O/CH_3CN (5:1) and UV detector was 228 nm.

Plant material

The wood of *T. yunnanensis* was collected from Mt. Laojunshan at an altitude of 3800 m, 100 km west of Lijiang City, Yunnan Province of the People's Republic of China in October 2000. A voucher sample (TMPW 21495) is preserved in the Museum of Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and isolation

The dried wood of *T. yunnanensis* (850 g) was extracted with H_2O (4 l × 3) under reflux for 30 min to yield an H_2O extract (52.2 g). The residue was further extracted with MeOH/H_2O (1:1) (4 l × 3) and MeOH (4 l × 3) to give MeOH/H_2O (32.2 g) and MeOH extracts (7.2 g), respectively. The H_2O extract, which had significant blood glucose lowering effect, was further divided into EtOAc soluble (34.1 g) and insoluble (16.1 g) fractions. The EtOAc soluble fraction gave three lignans, isotaxiresinol (**1**, 7.84 g), secoisolariciresinol (**2**, 3.63 g) and taxiresinol (**3**, 840 mg). These compounds were pure in TLC and ^{1}H-NMR spectral observations and their spectral data were identical with those in literatures (King et al., 1952; Agrawal and Rastogi, 1982; Mujumdar et al., 1972).

Animals

Male Wistar rats (5–6-weeks-old, 150–250 g) were used for present study. All the animals were purchased from Sankyo Labo Service (Tokyo, Japan) and were maintained on a 12 h light/dark cycle in a temperature and humidity controlled room. The animals were fed with a laboratory pellet chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water ad libitum during the experiment. This study was conducted in accordance with the standards outlined in the Guidelines for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

Diabetic model and treatment of extracts and compounds

Rats were made diabetic by a little modification of previously described method (Basnet et al., 1994). In brief, diabetes mellitus was introduced by a single intraperitoneal dose (55 mg/kg) of STZ dissolved in citrate buffer (pH 4.5) into 16 h-fasted rats. At the fifth day after STZ-injection, the rats were fasted 6 h and blood was taken from tail vein of anesthetized rats using a capillary tube. By centrifuging for 10 min (3000 rpm), plasma was separated from blood and the glucose level was measured by an enzymatic method using a glucose detecting kit; rats were considered diabetic when their fasting glycemia was >250 mg/dl. The diabetic rats were divided randomly into different groups consisting of four animals each and treated (i.p.) with extract or compound **1**–**3**. The group treated with a mixture of tolbutamide (200 mg/kg) and buformin (1 mg/kg) was taken as a positive control, while the group of rats treated with an equal volume of physiological saline and 2% acacia gum was taken as a negative control. The group without STZ treatment and treated with an equal volume of physiological saline and 2% acacia gum during the experiment was taken as a solvent control (normal). The extract or compound was administered at 9:00 and 17:00 on the first and second days and at 9:00 on the third day. From 9:00 of the third day, rats were fasted and the blood samples were collected at 15:00 from the tail vein. The decrease of blood glucose level was calculated as follows: % decrease in glucose $= [(G_0 - G_x)/G_0] \times 100$, where G_0 and G_x are the values of initial glycemia and the glycemia after

treatment. In all the experiments, the drugs were suspended in 2% acacia gum (w/v). The data are shown as mean ± S.E. and statistical significance was evaluated by unpaired Student's *t*-test.

Results and discussion

The wood of *T. yunnanensis* was extracted with H_2O under reflux for 30 min to give a H_2O extract (yield, 6.1%). The residue was further extracted with MeOH/H_2O (1:1) and MeOH to give MeOH/H_2O (3.8%) and MeOH (0.9%) extracts, respectively. TLC analysis of the latter two extracts showed that they are identical, and thus the blood glucose lowering effects of the H_2O and MeOH extracts were tested in STZ-induced diabetic rats. Both extracts were administered five times at a dose of 100 mg/kg (i.p.). The fasting blood glucose level of diabetic rats treated with the H_2O extract was significantly lowered as compared to that of the negative control group treated with equal volume of 2% acacia gum (Fig. 1). The blood glucose level of the group treated by the H_2O extract was lowered to 255.6 ± 39.1 mg/dl (33.7% reduction) from 385.7 ± 12.8 mg/dl by the administration. The blood glucose lowering effect of the H_2O extract at a 100 mg/kg dose was stronger than the mixture of tolbutamide (200 mg/kg), a known sulfonylurea hypoglycemic agent, and buformin (1 mg/kg), which was used as a positive control, and lowered the blood glucose level by 24.0%. The blood glucose levels of the rats treated only with STZ (negative control group) and of the solvent control (normal) rats remained steady at the same time interval (Fig. 1). The blood glucose level of diabetic rats treated by the MeOH extract (100 mg/kg, i.p.), on the other hand, slightly increased from 328 ± 10.0 to 350.6 ± 25.0 mg/dl. Thus, the H_2O extract of the wood of *T. yunnanensis* should have significant blood glucose lowering effect on diabetic rats. To the best of our knowledge, this is the first report of the hypoglycemic effect of the wood of *T. yunnanensis*.

The H_2O extract was then divided into EtOAc soluble and insoluble fractions, and from the former fraction three lignans, isotaxiresinol (**1**), secoisolariciresinol (**2**) and taxiresinol (**3**) (Fig. 2), were isolated by column chromatography (Banskota et al., 2003). Their yields were 0.9776%, 0.6526% and 0.1053%, respectively, with respect to the dried plant material, while HPLC analysis of the H_2O extract using a reversed-phase octadesylsilanized (ODS) column indicated that **1** and **2** were major compounds in the H_2O extract (Fig. 3a) of the wood. On the other hand, the major component of the MeOH extract was not lignans (Fig. 3b). The hypoglycemic effect of the major lignans **1–3** was then tested in the same experimental model at a dose of 100 mg/kg (i.p.). Compounds **1–3** significantly lowered the blood glucose level of diabetic rats after five times treatment. The blood glucose level of the group treated by **1** was reduced to 236.9 ± 33.4 (34.5% reduction) from the initial value of 361.5 ± 12.3 mg/dl, while that of the group treated by **2** became 236.9 ± 23.8 (33.4% reduction) from 355.9 ± 17.1 mg/dl (Fig. 4). The blood glucose lowering effects of **1** and **2** were stronger than the positive control. Taxiresinol (**3**), on the other hand, lowered the fasting blood glucose level of the diabetic rats by only 20.9%. These results led us to conclude that these major lignans **1–3** would be responsible for the blood glucose lowering effect of the H_2O extract of the wood. The blood glucose lowering effect of the major lignans was either equal to or lower than the H_2O extract, indicating that there should be some synergistic effect, which is common in traditional or crude drugs.

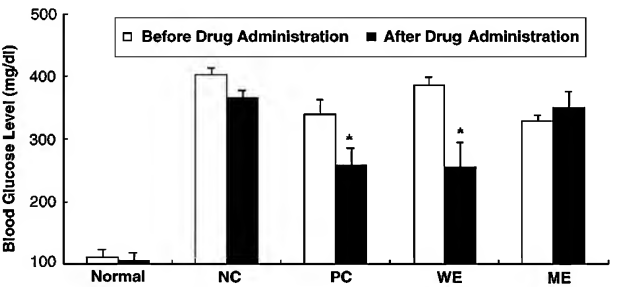

Fig. 1. Hypoglycemic activities of the H_2O and MeOH extracts of *T. yunnanensis* on STZ-induced diabetic rats. In each group four rats were taken. $^*p < 0.05$ significantly different from the STZ-treated negative control group. Solvent control (normal), without STZ treatment; NC, treated by an equal volume of physiological saline and 2% acacia gum (negative control); PC, positive control treated with the mixture of tolbutamide (200 mg/kg, i.p.) and buformin (1 mg/kg, i.p.); WE, treated by the H_2O extract (100 mg/kg, i.p.); ME, treated by the MeOH extract (100 mg/kg, i.p.).

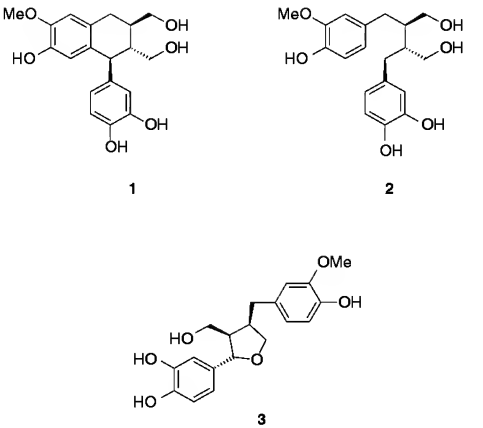

Fig. 2. Structure of lignans **1–3** isolated from the wood of *T. yunnanensis*.

STZ is widely used for induction of experimental diabetes mellitus, because of toxic effect to pancreatic β-cells, which is responsible for the secretion of insulin (Like and Rossini, 1976). The excessive production of reactive oxygen species (ROS) including nitric oxide (NO) and subsequent increase of local oxidative stress is suggested as one of the pathophysiological mechanisms of STZ-induced diabetes mellitus (Haluzik et al., 1998; Wilson et al., 1984; Corbett and McDaniel, 1992). Therefore, antioxidants to reduce oxidative stress by inhibiting ROS were considered to be promising agents against STZ-induced diabetes mellitus (Baynes, 1991; Robbins et al., 1980; Chowienczyk et al., 2000). In our previous study, we already demonstrated that the H_2O extract and major lignans **1–3** possess potent radical scavenging properties toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and significant inhibitory activity against lipopolysaccharide (LPS)-induced NO production in murine macrophage-like J774.1 cells (Banskota et al., 2003). Thus, the hypoglycemic properties of the wood of *T. yunnanensis* and the isolated lignans may directly correlate with their antioxidative properties. Although secoisolariciresinol diglucoside (SDG) was previously reported to have protective effect against STZ-induced diabetes mellitus by reducing oxidative stress (Prasad, 2000; Prasad et al., 2000), this is the first report of hypoglycemic effect of secoisolariciresinol (**2**), aglycone of SDG, and taxiresinol (**3**) on STZ-induced diabetic rats. On the other hand, isotaxiresinol (**1**) was reported to have significant hypoglycemic effect at 100 mg/kg in rats (Guo et al., 1994).

Lignans were found in a variety of foods, vegetables and fruits, and were reported to have potential

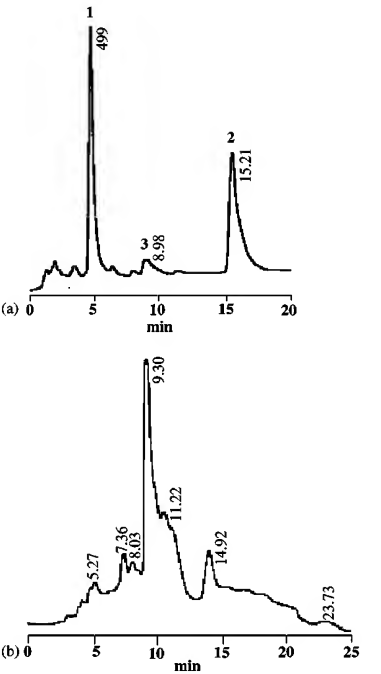

Fig. 3. HPLC chromatogram of the H_2O (a) and MeOH (b) extracts of *T. yunnanensis*. HPLC was performed in Shimadzu LC-6AD using discovery C18 column (4.6 mm i.d. × 25 cm). Mobile phase was H_2O/CH_3CN (5:1) 2 ml/min, injection volume 10 µl (5 mg/ml) and UV detector was 228 nm.

protective effects on human health. Because of their possible function as weak estrogenous or estrogen antagonists, they are also called phytoestrogens (Nicolle et al., 2002). Both experimental and epidemiological studies suggest that high plasma and urinary concentrations of phytoesterogens, including lignans, are associated with decreased risk for hormone-dependent diseases, e.g., breast cancer and coronary heart diseases (Vanharanta et al., 1999; Ingram et al., 1997). Moreover, in a recent report, Bhathena and Velasquez (2002) described that dietary phytoestrogens, i.e., soy isoflavones and flaxseed, improves glucose control and insulin resistance in human and animals. However, it is not clear what compounds are responsible for such action. The present study suggests that dibenzylbutane [SDG or secoisolariciresinol (**2**)] or aryltetralin-type [isolariciresinol or isotaxiresinol (**1**)] lignans may contribute for such interesting activity of flaxseed, which is rich in lignans. The present study further notices that the wood of

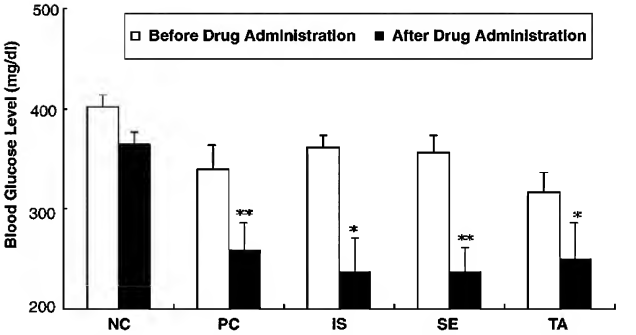

Fig. 4. Hypoglycemic activity of lignans **1**–**3** on STZ-induced diabetic rats. In each group four rats were taken. $^{**}p<0.01$, $^{*}p<0.05$ significantly different from the STZ-treated negative control group. NC, treated by an equal volume of physiological saline and 2% acacia gum (negative control); PC, positive control treated with the mixture of tolbutamide (200 mg/kg, i.p.) and buformin (1 mg/kg, i.p.); IS, treated by **1** (100 mg/kg, i.p.); SE, treated by **2** (100 mg/kg, i.p.); TA, group treated by **3** (100 mg/kg, i.p.).

T. yunnanensis became a potent source of secoisolariciresinol (**2**), which is considered as an important precursor of mammalian lignan (Axelson et al., 1982).

In conclusion, from the water extract of *T. yunnanensis*, showing the potent hypoglycemic effect on STZ-induced diabetic rats, three lignans, isotaxiresinol (**1**), secoisolariciresinol (**2**) and taxiresinol (**3**), were isolated as main constituents corresponding to the effect. The hypoglycemic effect of these lignans was considered to associate with their antioxidative properties. Even though further studies on oral administration, on mechanistic aspect and on minor substances should be needed, the present results would provide the scientific evidence of traditional uses of this plant for treatment of diabetes mellitus in different ethnic societies of Yunnan Province of China.

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